"Integration of a Micro-Chip Amino Acid Chirality Detector into the MOD Instrument Concept"
Final Report
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PI: Jeffrey L. Bada, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212

Co-PI; Frank Grunthaner, Device Research and Applications Section, NASA Jet Propulsion Laboratory, Pasadena, CA 91109

Co-PI; Richard Mathies, Department of Chemistry, University of California, Berkeley, CA 94720

Summary: The MOD (Mars Organic Detector) instrument concept consists of a sublimation apparatus for organic compound isolation connected to a microfabricated microfluidic analyzer containing a sipper, pumps and a separation channel for organic compound characterization. The target organic compounds are amino acids and polycyclic aromatic hydrocarbons (PAHs). Solid samples are placed within the sublimation apparatus and heated to release organic compounds which sublime onto a cold finger. Half of the cold finger is coated with fluorescamine, which reacts with amino acids and other primary amines to generate an intense fluorescent derivative while the other half is uncoated and is used to directly detect PAH fluorescence. A capillary sipper is then used to dissolve and sample the labeled amino acids and integrated microfabricated pumps transport the labeled amino acids to the chip for analysis. The sample is separated using capillary zone electrophoresis (CZE) together with chiral dextrins to determine amino acid composition and chirality. During the grant period, the following steps have been completed toward the development of a robust instrument and chemistry.

A) Basic MOD Instrument

The basic MOD instrument consists of a sublimation cell, a chemical detector, and a fluorescence analyzer (see **Figure 1**). During the operational sequence, a sample delivery system transfers a soil or crushed rock sample to the MOD oven for analyses.

After closing the oven at Mars ambient pressure, the sample is stepwise heated to 950°C. Amino acids and PAHs in the sample sublime and are collected on a cold finger that is cooled to Mars nighttime temperatures (around -100°C). The MOD cold finger is divided into two zones: one is coated with fluorescamine which reacts with amino acids and other primary amines to generate an intense fluorescent derivative; the other is uncoated and is used to directly detect PAHs, which do not require a reagent in order to produce an intense UV fluorescent signal. The sublimed target compounds are detected using laser-based fluorescence sensors with sensitivities in the 10 ppt range, which is at least 1000X more sensitive than the Viking GCMS.

B) Separation of Fluorescamine-Labeled Amino Acids:

The CZE-based separation of fluorescamine-labeled amino acids has been studied to determine the optimal conditions for resolution of a standard mixture (1). The amino acids in the standard (valine, AIB, alanine, serine, glycine, glutamic acid, aspartic acid) are most prominent in the Murchison meteorite. The effect of buffer concentration, pH and temperature were studied, and it was found that an 8 -10 mM CO_3^{2-} pH 8.8 buffer, run at 8 °C, provided optimal separation (**Figure 2A**).

The chiral separation of fluorescamine-labeled amino acids was also studied to optimize the type of cyclodextrin and concentration, the buffer concentration and pH, and the temperature. The best resolution of the different enantiomers present in the standard was obtained using a hydroxy-propyl-β-cyclodextrin buffer at pH 8.8, and the lowest temperature (4 °C) possible without condensation on the chip surface. All stereoisomers formed in the labeling reaction of the chiral dye with the chiral amino acids are typically resolved (**Figure 2B**).

Typical limits of detection are ~50 nM. These results demonstrate the feasibility of combining fluorescamine labeling of amino acids with microfabricated CE devices to develop low-volume, high-sensitivity apparatus and methods for extraterrestrial exploration.

C) Sampling from the MOD Cold Finger:

Several experiments were performed using a microfrabricated sipper (**Figure 3**) to collect portions of extracts prepared by subliming amino acid solutions of known

concentration onto a disk coated with fluorescamine using the MOD sublimation apparatus. Buffer was manually expelled onto the disk to pick up fluorescamine-labeled amino acids and then transported to the μCE device for analysis. This work established the feasibility of the method for interfacing the sublimation apparatus with the microfabricated analyzer in the basic MOD instrument design.

D) Development of a field unit and testing under field conditions:

A portable field version of the amino acid analysis device has developed and this was successfully integrated with the MOD sublimation apparatus in the field. This effort required the development and characterization of monolithic elastomer membrane valves and diaphragm pumps suitable for large-scale integration into glass microfluidic analysis devices (2, 3). Valves and pumps are fabricated by sandwiching an elastomer membrane between etched glass fluidic channel and manifold wafers. A three-layer valve and pump design features simple non-thermal device bonding and a hybrid glass-PDMS fluidic channel; a four-layer structure includes a glass fluidic system with minimal fluid-elastomer contact for improved chemical and biochemical compatibility. The pneumatically actuated valves have <10 nL dead volumes, can be fabricated in dense arrays, and can be addressed in parallel via an integrated manifold. The membrane valves provide flow rates up to 380 nL/s at 30 kPa driving pressure and seal reliably against fluid pressures as high as 75 kPa. The diaphragm pumps are self-priming, pump from a few nL to a few microliters per cycle at overall rates from 1-100 nL/s, and can reliably pump against 42 kPa pressure heads. These valves and pumps provide a facile and reliable integrated technology for fluid manipulation in complex glass microfluidic and electrophoretic analysis devices. This technology was the key for the development of the integrated amino acid analysis devices.

The next phase of the development (4, 5) involved the construction of the Mars Organic Analyzer (MOA), a portable version of the microfabricated capillary electrophoresis (CE) instrument (**Figure 4**). This microdevice consists of a four-wafer sandwich combining glass CE separation channels, microfabricated pneumatic membrane valves and pumps, and a nanoliter fluidic network. The portable MOA instrument integrates high voltage CE power supplies, pneumatic controls, and fluorescence detection optics necessary for field operation. The amino acid concentration sensitivities

range from μM to 0.1 nM corresponding to part-per-trillion (pptr) sensitivity. The MOA is first used in the lab (6) to analyze soil extracts from the Atacama Desert, Chile, detecting amino acids ranging from 10 to 600 ppb. Field tests of the MOA in the Panoche Valley, CA (6) successfully detected amino acids at the 70 pptr to 100 ppb levels in jarosite (**Figure 5**), a sulfate-rich mineral associated with liquid water that was recently detected on Mars. These results demonstrate the feasibility of using the MOA to perform highly-sensitive *in situ* amino acid biomarker analysis on soil samples representative of a Mars-like environment. More complete details and images of all of our field work will be found at http://www.astrobiology.berkeley.edu.

E) Concept for the next generation instrument:

We have initiated the development of a more general chemical analysis microdevice which is called the Mars Organic Laboratory (7). The idea is to exploit our microfluidic and integration technologies to develop analyzers for broader classes of organic molecules of potential biological relevance. In particular the basic ideas and microchips needed to detect zwitterionic amino acids as well as nucleobases, sugars, organic acids and organic bases using novel capture matrix chemistries and chambers is developed. The central idea is to perform two-dimensional analysis of analytes. The first dimension is the affinity dimension where input molecules are captured, concentrated and purified according to unique chemical functional groups. This separates the molecules into the classes defined above. Next, the target molecules are released, labeled and electrophoretically separated to produce a molecular chromatogram indicative of size.

Figure 1: The basic MOD design.

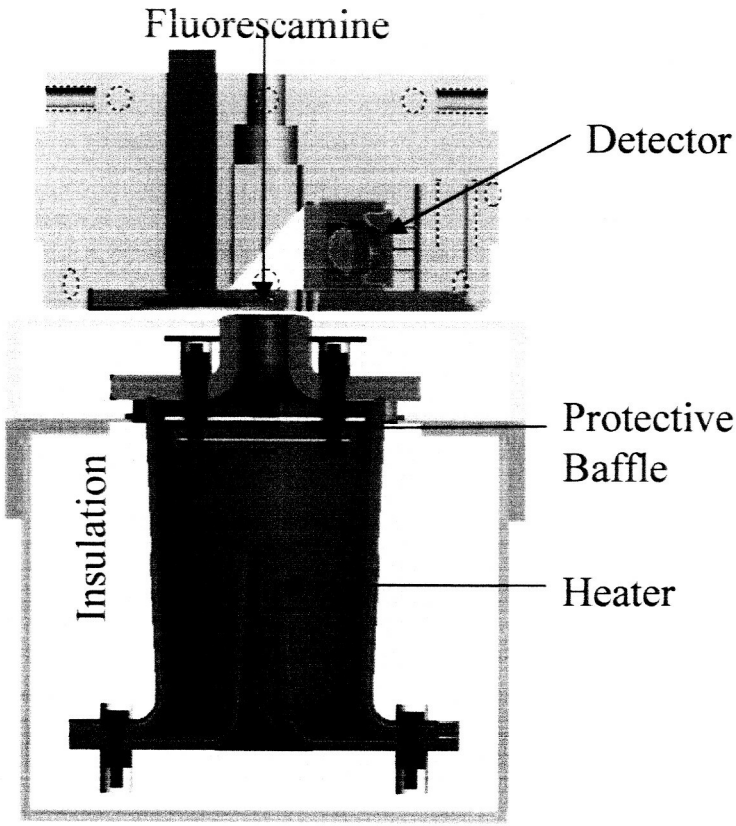


Figure 2: Analysis of sublimation samples from the MOD cold finger. Samples were collected by manually pipetting buffer onto chip surface, collecting, and diluting as indicated. (A) The composition of the samples was determined in a buffer containing 8 mM CO_3^{2-} at pH 8.8. (B) The chiral separations were performed by adding 15 mM HPβCD to the buffer.

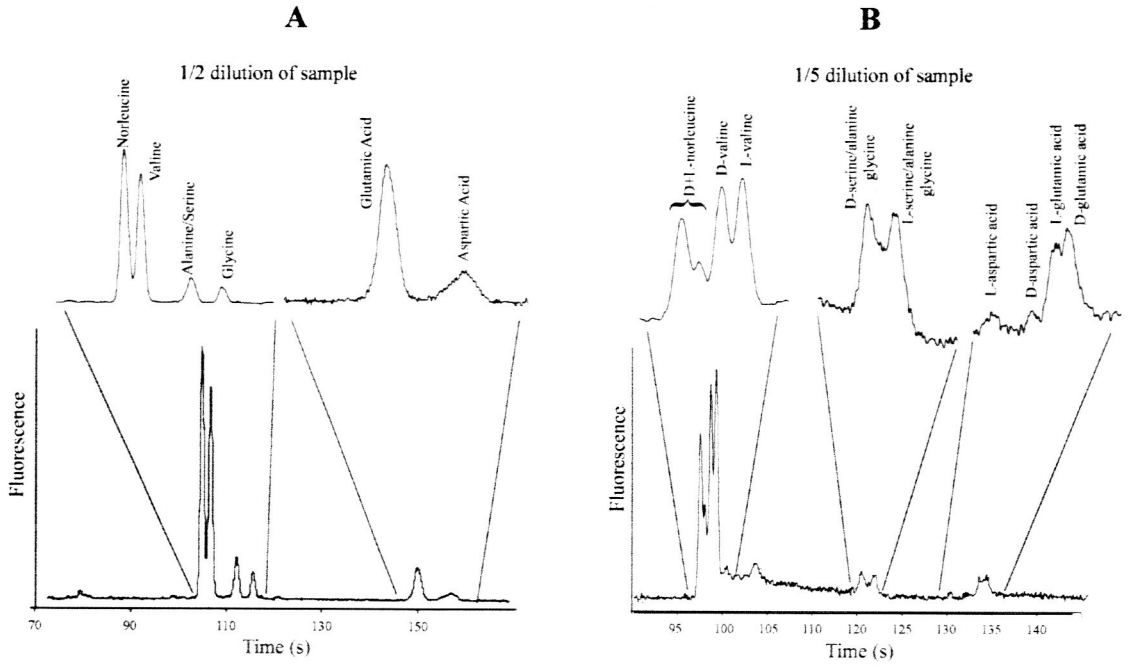


Figure 3: Microfabricated device designed to interface with MOD cold finger for amino acid analysis. Sample obtained from the MOD cold finger is drawn in through the sipper by a microfabricated pump operated by an integrated pneumatic manifold and pumped to the sample reservoir for CE.

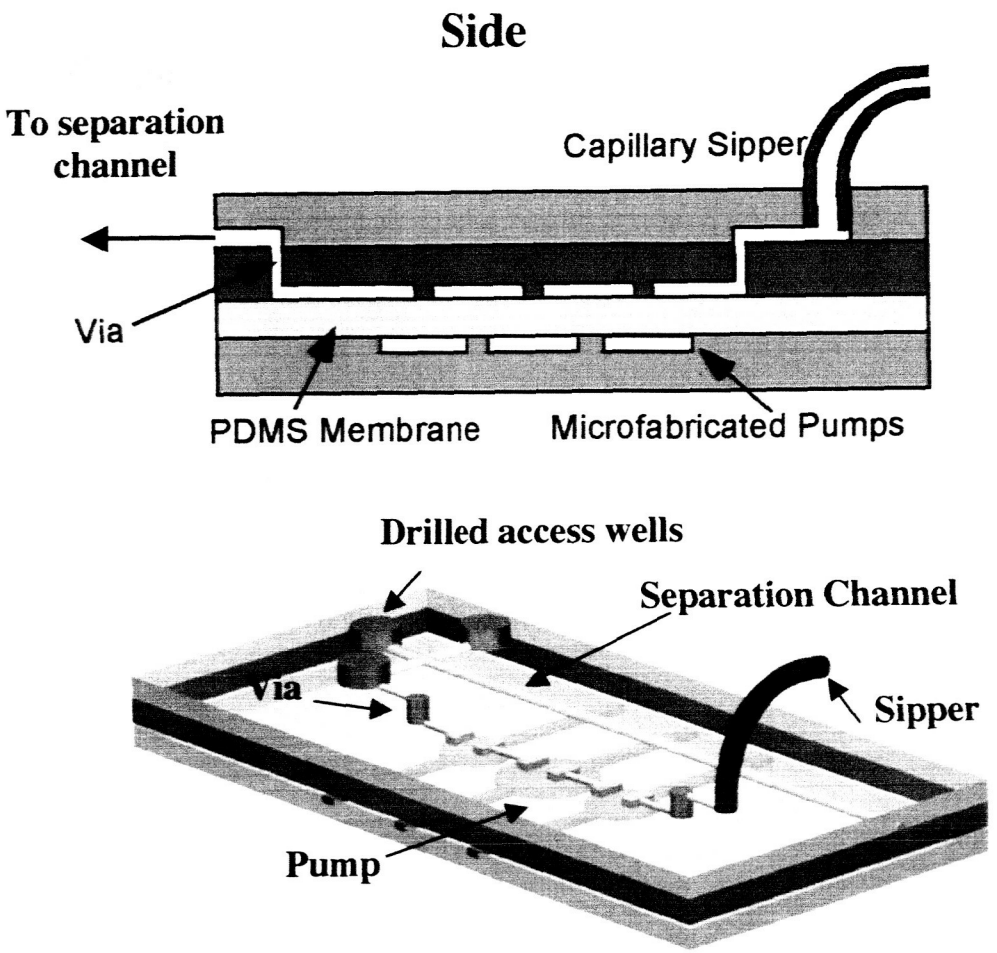


Figure 4: Photograph of the Mars Organic Analyzer or MOA. This portable analysis device integrates all functions necessary for field analysis of amino acids including the potentials for the microchip electrophoresis, pneumatic actuation of microfluidics, laser excitation and detection, and chip temperature control.

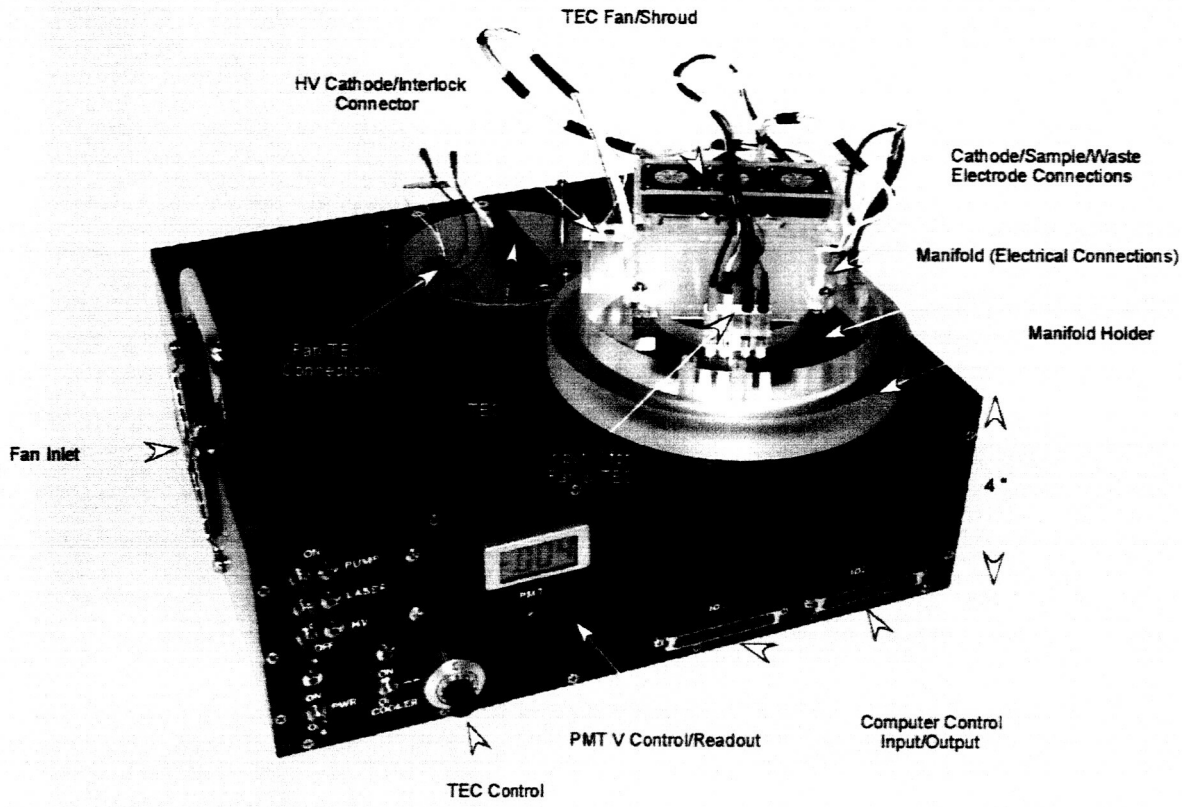
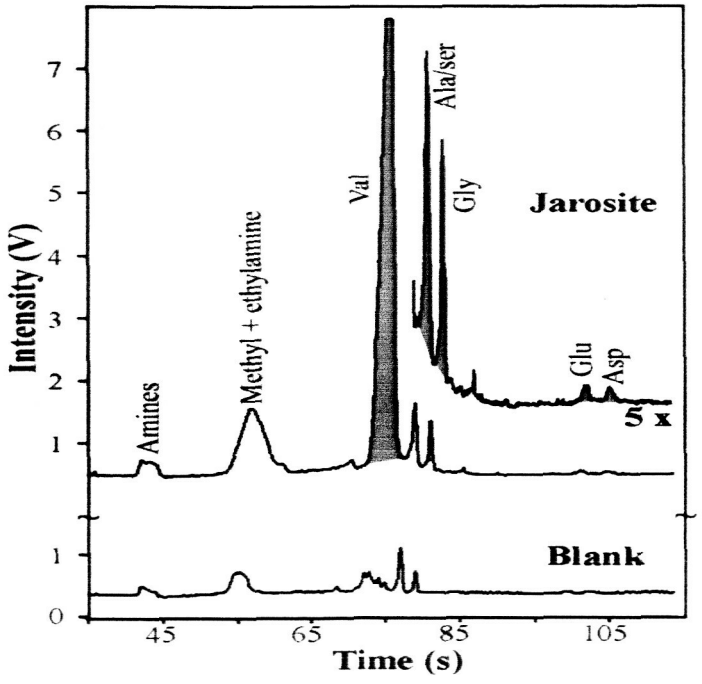


Figure 5: Analysis of amino acids from a sample of jarosite performed in the Panoche Valley CA by the MOA. These traces were obtained by sampling labeled amino acids from the MOD sublimation device and performing electrophoretic separation. These jarosite samples are found to contain up to 100 ppb concentrations of amino acids above the blank.



Publications supported by this grant

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3. Grover, W. H., Skelley, A. M., Liu, C. N., Lagally, E. T., and Mathies, R. A. Practical Valves and Pumps for Large-Scale Integration in Microfluidic Devices, *Micro Total Analysis Systems 2002*, Volume 1, eds. Y. Baba, S. Shoji and A. van den Berg, Kluwer Academic (Dordrecht, The Netherlands), pp 136-138.

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5. Skelley, A. M., Scherer, J. R., Aubrey, A. D., Ivester, R. H. C., Ehrenfreund, P., Grunthaner, F. G., Bada, J. L. and Mathies, R. A. Sensitive Amino Acid Composition and Chirality Analysis with the Mars Organic Analyzer (MOA), *Proceedings of the 55th International Astronautical Congress*, 10/4-10/8/04, Vancouver, Canada (2004).

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7. Skelley, A. M., Grunthaner, F. J., Bada, J. L., and Mathies, R. A. Mars Organic Detector III: A Versatile Instrument for Detection of Bio-organic Signatures on Mars, in SPIE Vol. 4878 *First Jet Propulsion Laboratory In Situ Instruments Workshop*, ed. By G. H. Bearman, P. M. Beauchamp (SPIE. Bellingham, WA 2003), pp. 59-67.